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Ketamine in the treatment of depression: clinical utility, safety, and mechanism of action

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KETAMINE IN THE TREATMENT OF DEPRESSION: CLINICAL UTILITY, SAFETY, AND MECHANISM OF ACTION

by

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NAKUL VYAS

ABSTRACT

Ketamine has shown promise as a novel treatment for depression and as a means to investigate the biology of depression. The drug effectively and rapidly treats depressed patients with the effects lasting approximately 1 week. However, concerns about ketamine's efficacy do exist because of the inadequacy of blinding procedures used in existing trials. A dose of 0.5 mg/kg has been found to be most effective. Prolonged ketamine infusions have not extended the antidepressant effect beyond the timeframe of a regular infusion. Repeat infusions may be successful in extending ketamine's effect, but definite conclusions cannot yet be made in this regard. Combination treatment with escitalopram and cognitive behavioral therapy (CBT) hold promise, as does the development of an intranasal formulation. Ketamine has shown additional efficacy as an acute anti-suicide treatment. Side effects from a single administration usually fade within a few hours and commonly include dissociation, elevations of blood pressure, nausea, and anxiety. Less data is available on the side effects caused by repeated ketamine infusions. Concerns exist regarding genitourinary, hepatic, and cognitive side effects after repeated infusions, as well as a risk of addiction. Research on ketamine's mechanism of action has focused on the glutamate system in the brain. Ketamine may act by inhibiting release of γ–aminobutyric acid (GABA) from interneurons, activating intrasynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), increasing

mammalian target of rapamycin complex 1 (mTORC1) and extracellular signal-regulated kinase (ERK) signaling, enhancing brain-derived neurotrophic factor (BDNF) production, inhibiting glycogen synthase kinase 3 (GSK3), blocking extrasynaptic *N*-Methyl-D-aspartate receptors (NMDARs), and promoting synaptogenesis and neuroplasticity. The two existing ketamine stereoisomers, (*R*)- versus (*S*)-ketamine, have different actions and potentially different efficacies and side effect profiles. Ketamine also produces regional changes in brain activity and connectivity. These include decreased burst firing in the lateral habenula (LHb), increased activity in the prefrontal cortex (PFC) and subgenual anterior cingulate cortex (ACC), and alterations in the amygdala's response to angry and happy faces. Ketamine has the potential to be developed into a novel and useful clinical tool in the treatment of depression and to advance the understanding of the biology of depression.

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LIST OF ABBREVIATIONS

^{1}H-MRS .....proton magnetic resonance spectroscopy

4E-BP1 .....eukaryotic translation initiation factor 4E-binding protein 1

ACC ..... anterior cingulate cortex

ACPC ..... 1-aminocyclopropanecarboxylic acid

Akt ..... protein kinase B

AMPA .....α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR ..... α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

AP-7 .....2-amino-7-phosphonoheptanoic acid

Arc ..... activity-regulated cytoskeleton-associated protein

BDNF ..... brain-derived neurotrophic factor

BOLD ..... blood oxygen level-dependent

CaMKII ..... Ca^{2+}/calmodulin dependent protein kinase II

cAMP ..... cyclic adenosine monophosphate

CBT .....cognitive behavioral therapy

CREB ..... cyclic adenosine monophosphate response element-binding protein

EAAT .....excitatory amino acid transporter

ECT ..... electroconvulsive therapy

eEF2 ..... eukaryotic elongation factor 2

eEF2K ..... eukaryotic elongation factor 2 kinase

eIF4E .....eukaryotic translation initiation factor 4E

ERK .....extracellular signal-regulated kinase

GABA .....γ–aminobutyric acid

$GABA_A$ .....γ–aminobutyric acid A receptor

Gln .....glutamine

GLS ..... glutaminase

Glu .....glutamate

GluA1 .....glutamate receptor 1 subunit

GluR1 .....glutamate receptor 1 subunit

GSK3 .....glycogen synthase kinase 3

HDRS ..... Hamilton Depression Rating Scale

HNK .....(2R,6R)-hydroxynorketamine

LHb ..... lateral habenula

MADRS ..... Montgomery-Åsberg Depression Rating Scale

MAPK ..... mitogen-activated protein kinase

MDD ..... major depressive disorder

MEK .....mitogen-activated protein kinase kinase

mGluR .....metabotropic glutamate receptor

MK-801 .....dizocilpine

mTOR ..... mammalian target of rapamycin

mTORC1 .....mammalian target of rapamycin complex 1

NMDA .....*N*-Methyl-D-aspartate

NMDAR .....*N*-Methyl-D-aspartate receptor

p70S6K .....ribosomal protein S6 kinase beta-1

PFC .....prefrontal cortex

P_i ..... inorganic phosphate

PKA ..... protein kinase A

PKC ..... protein kinase C

PI3K .....phosphoinositide 3-kinase

PSD95 ..... post-synaptic density protein 95

QIDS-SR ..... Quick Inventory of Depressive Symptomatology–Self Report

RAS-MAPK .....RAS-mitogen-activated protein kinase

RCT .....randomized control trial

TNFR1 ..... tumor necrosis factor receptor 1

TRKB ..... tropomyosin-related kinase B

VDCC .....voltage-dependent calcium channel

VGLUT .....vesicular glutamate transporter

xCT .....cysteine-glutamate antiporter

BACKGROUND

In a given year in the United States, about 9.1% of the population experiences an episode of major depression. An additional 2.3% will experience bipolar depression in the same year. Over a lifetime, an estimated 16.8% of the U.S. population experiences major depression and 3.1% experiences bipolar depression (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Depression generates significant economic burden and disability. Unfortunately, several weeks are usually needed for current treatments to take effect. In addition, two-thirds of major depression patients do not respond to the medication initially prescribed (Li et al., 2010; Zarate et al., 2012).

Depression is also a prime risk factor for suicide. Among individuals that commit suicide, 90% have a psychiatric disorder. The psychiatric disorder is most often a mood disorder. Suicide is one of the top three causes of mortality worldwide for individuals aged 15 – 44 (Wilkinson, Ballard, et al., 2017). Of further concern, the suicide rate in the United States has been rising steadily since 2000 (Hedegaard, Curtin, & Warner, 2018). Current treatment options for acute suicide risk are limited. These treatment options most commonly consist of hospitalization, pharmacotherapy, psychotherapy, electroconvulsive therapy (ECT), or a combination thereof. Lithium, dialectical behavior therapy, and cognitive behavioral therapy (CBT) have been shown to reduce suicide deaths over the long-term, but have not been demonstrated to reduce deaths during acute treatment (Wilkinson, Ballard, et al., 2017).

In this context, ketamine's rapid onset of action and effectiveness in patients who have not responded to other treatments have generated considerable interest (Li et al., 2010; Wilkinson, Ballard, et al., 2017). Ketamine has also generated excitement because the drug's mechanism was thought to involve the glutamate system in the brain, rather than the monoamine systems that historically have been the major focus in treating depression (Sanacora, Zarate, Krystal, & Manji, 2008). Before turning to early studies on anti-depressant agents that act on the glutamate system, the operation of glutamate signaling in the brain will be outlined first. Then alterations of glutamate signaling in depressed patients will be catalogued.

Overview of Glutamate Signaling

Glutamate is the brain's main excitatory neurotransmitter. Glutamate binds ionotropic receptors, which occur in three types: kainate receptors, *N*-Methyl-D-aspartate receptors (NMDARs), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs; **Figure 1**). The binding of glutamate to these ionotropic receptors causes short-term changes in the post-synaptic cell's membrane potential. Glutamate also binds metabotropic glutamate receptors (mGluRs), which alter processes within the cell through G proteins. Binding of glutamate to both ionotropic and metabotropic receptors can cause longer-term changes in synaptic strength through alterations in second messenger systems, transcription, and translation.

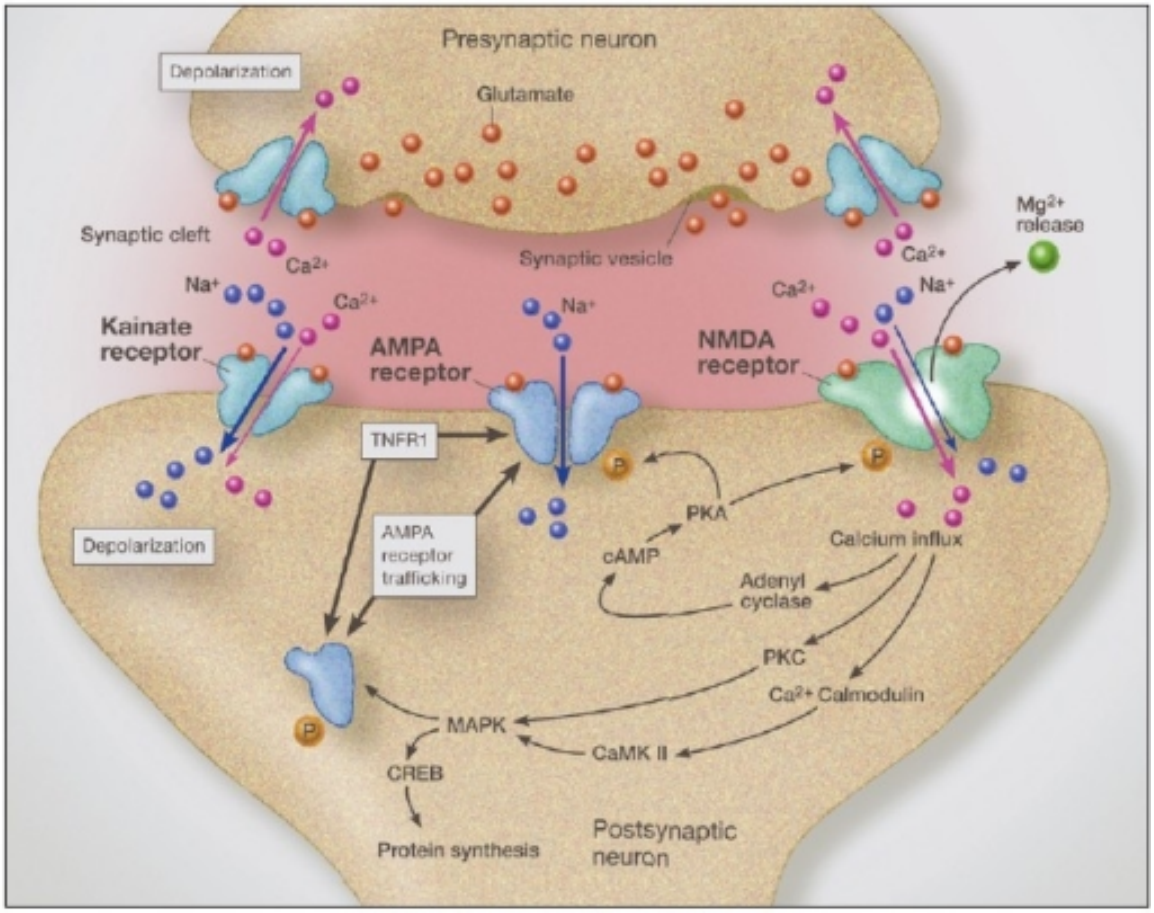

Figure 1: Glutamate Synapse. Three types of ionotropic glutamate receptors are present in the nervous system: kainate receptors, AMPARs, and NMDARs. When glutamate binds to these receptors, they respond by opening their ion channels. All three receptor ion channels are permeable to Na^+ ions. The NMDAR ion channel is distinguished by its capacity to also allow a large number of Ca^{2+} ions to cross the plasma membrane. Calcium influx into the post-synaptic cell can, in turn, activate a number of second messenger systems that promote cell survival, protein synthesis, and AMPAR insertion into the plasma membrane. *AMPAR*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; *CaMK II*, Ca^{2+}/calmodulin-dependent protein kinase II; *cAMP*, cyclic adenosine monophosphate; *CREB*, cyclic adenosine monophosphate response element-binding protein; *MAPK*, mitogen-activated protein kinase; *NMDAR*, *N*-Methyl-D-aspartate receptor; *PKA*, protein kinase A; *PKC*, protein kinase C; *TNFR1*, tumor necrosis factor receptor 1. Figure from (Blaylock & Maroon, 2011).

Though AMPARs, kainate receptors, and NMDARs have different channel structures, the opening of all three receptor channels permits Na^+ to enter the cell. NMDARs are unique in that most of the inward current through the ion channel is carried by Ca^{2+}. AMPARs are composed of four glutamate receptor subunits: GluR1 - GluR4. Glutamate binding to at least two of these subunits induces channel opening. Kainate receptors are tetramers composed of a combination of five possible subunits: GluR5, GluR6, GluR7, KA1, and KA2. NMDARs are composed of two NR1 and two NR2 subunits. For the NMDAR channel to open, three actions must occur: the co-agonist glycine must bind to each of the NR1 subunits, glutamate must bind to each of the NR2 subunits, and the Mg^{2+} atom that blocks the channel pore at rest must be displaced by initial depolarization through other receptors. Often AMPARs are responsible for this initial depolarization, which removes the Mg^{2+} from the NMDAR pore (Murrough, Abdallah, & Mathew, 2017).

The multiple requirements for opening the NMDAR channel endow the NMDAR with the capacity to serve as a "coincidence detector." In its role as a coincidence detector, the NMDAR opens in response to multiple converging excitatory inputs on the neuron. Entry of calcium into the neuron through the NMDAR channel then activates second messenger pathways, including Ca^{2+}/calmodulin dependent protein kinase II (CaMKII). Activation of CaMKII causes insertion of AMPARs into the plasma membrane (Murrough et al., 2017). Additional AMPARs in the membrane make the postsynaptic cell more likely to depolarize in response to subsequent stimulation

(Malenka & Nicoll, 1999). This strengthening of the synapse is called long-term potentiation (Malenka & Bear, 2004).

NMDAR signaling can promote cell survival or stimulate apoptosis depending on the duration of receptor activation and the location of the NMDAR relative to the synapse. Moderate NMDAR activation is neuroprotective, activating the RAS-mitogen-activated protein kinase (RAS-MAPK) and cyclic adenosine monophosphate response element-binding protein (CREB) pathways. RAS-MAPK and CREB induce genes for cell survival. Induction by CREB increases brain-derived neurotrophic factor (BDNF) expression. On the other hand, over-release of glutamate and prolonged activation of NMDARs is associated with neuronal excitotoxicity. Activation of NMDARs within the synapse is neurotrophic. In contrast, activation of extrasynaptic NMDARs favors apoptosis (Murrough et al., 2017).

Glial cells are also important modulators of glutamate signaling. Glial cells remove glutamate from the synapse through excitatory amino acid transporters (EAATs) and convert the glutamate to glutamine (**Figure 2**). Via glycine transporter 1, glial cells also remove glycine from synapses (Murrough et al., 2017). Astrocytes synthesize and release the trophic factors BDNF and glial-derived neurotrophic factor, which enhance maintenance of glutamatergic synapses and neuronal survival (Murrough et al., 2017; Sanacora & Banasr, 2013).

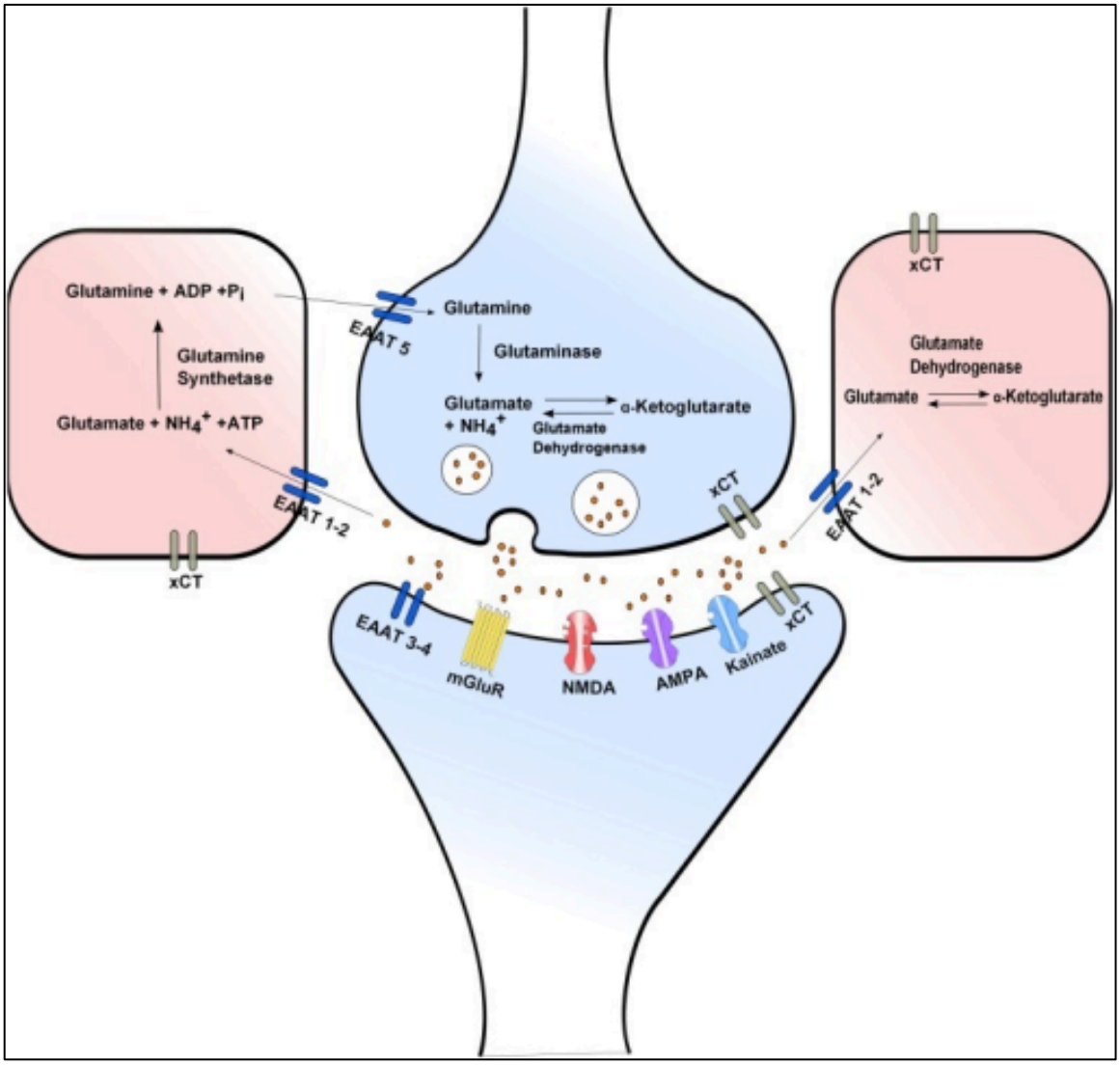

Figure 2: Glutamate/Glutamine Metabolism in Glial Cells Around Synapse. Glial cells, shown in peach, clear glutamate from the synapse through excitatory amino acid transporters (EAATs). The glial cells metabolize glutamate to glutamine using glutamine synthetase. The glutamine can then be transported to the pre-synaptic neuronal terminal, where the glutamine can be converted back to glutamate using glutaminase. The glutamate can then be released into the synapse to bind to receptors on the post-synaptic neuronal terminal. The pre-synaptic and post-synaptic neuronal terminals are shaded blue. *AMPA*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *EAAT*, excitatory amino acid transporter; *NMDA*, *N*-Methyl-D-aspartate; *mGluR*, metabotropic glutamate receptor; P_i, inorganic phosphate; *xCT*, cysteine-glutamate antiporter. Figure from (Kritis, Stamoula, Paniskaki, & Vavilis, 2015).

Alterations in Glutamate Transmission in Depression

Depressed patients show a number of abnormalities of glutamate signaling in the brain. Elevated glutamate has been found in fluid samples, genetic analyses, and post-mortem studies of depressed patients. Chronic stress alters glutamatergic transmission in both human and animal studies. Brain imaging research has also found alterations in glutamate signaling in various brain regions.

Serous fluid assays, post-mortem, and genetic studies all show evidence of glutamate system abnormalities that accompany depression. Plasma, cerebrospinal, and brain glutamate levels are increased in depressed patients. Additionally, a small number of genetic studies have shown an association between depression and particular genes related to glutamate signaling. Though there are some inconsistencies regarding the direction of effect in various brain regions, elevated glutamate has been found in post-mortem studies of depressed patients. Post mortem studies have also showed altered NMDAR function or expression in patients with major depressive disorder (MDD), bipolar disorder, and those who died from suicide (Murrough et al., 2017).

In animal and human studies, chronic stress is associated with depression. Animals subjected to chronic stress show altered glutamine release and reuptake. In these animals, glia take in less extracellular glutamate than normal, raising the extracellular glutamate concentration around neurons. The heightened extracellular glutamate increases activation of extrasynaptic NMDARs, whose activation in turn degrades synapse function and causes apoptosis of neurons (Murrough et al., 2013). In prefrontal cortex (PFC) neurons of rats under chronic stress, AMPAR and NMDAR subunits

decrease as a consequence of ubiquitin–proteasome-dependent degradation. Across the PFCs of these rats, synaptic spine density and dendritic complexity decreases (Popoli, Yan, McEwen, & Sanacora, 2012). The decreased intrasynaptic glutamatergic and increased extrasynaptic glutamatergic signaling associated with chronic stress also reduces BDNF signaling within hippocampal and cortical glutamatergic synapses. Patients with mood disorders have been shown to have fewer glia in their PFCs (Murrough et al., 2017). Animal studies also indicate that chronic stress may disrupt astrocyte function by reducing glial fibrillary associated protein, a component of astrocyte cytoskeletons (Banasr et al., 2010).

Elevated glutamate has been found in brain imaging of depressed patients. However, the direction of effect may differ across brain regions. Proton magnetic resonance spectroscopy (^{1}H-MRS) shows a reduced glutamate plus glutamine signal in the PFCs of patients with MDD. The same method found that γ–aminobutyric acid (GABA) levels are reduced in the PFC and occipital cortex, while glutamate levels in the occipital cortex are increased (Murrough et al., 2017). A study using ^{13}C magnetic resonance spectroscopy found that the oxidative energy production of glutamatergic neurons is reduced by 26% in patients with MDD without any decrease in glutamate-glutamine cycling (Abdallah et al., 2014).

Early Studies of NMDAR Antagonists

In the 1990s, a research group performed a preliminary set of experiments to assess whether NMDAR antagonists had antidepressant effects in animal models. Three

such agents were discovered to reduce mouse immobility in the forced swim test in a dose dependent fashion. These were dizocilpine (MK-801), an NMDAR channel blocker; 2-amino-7-phosphonoheptanoic acid (AP-7), a competitive antagonist at the NMDAR; and 1-aminocyclopropanecarboxylic acid (ACPC), an NMDAR glycine site partial antagonist. In follow-up experiments, imipramine, citalopram, and ECT were all found to affect transmission at NMDARs in a long lasting fashion. The group proposed that modulation of NMDARs might be the final common pathway of all antidepressants (Murrough et al., 2017).

The first clinical study to test whether ketamine had antidepressant effects in humans was a study in 2000 by Berman et al. (Murrough et al., 2017). As a preliminary study, the sample size was nine patients. Ketamine was found to significantly reduce Hamilton Depression Rating Scale (HDRS) scores in comparison to saline. Mood returned to pre-treatment baseline within one or two weeks. The individual HDRS items that underwent statistically significant improvement were depressed mood, helplessness, and worthlessness.

In this initial study (Berman et al., 2000), ketamine administration produced substantial, but transient cognitive deficits and euphoria. The euphoria or "high" returned to baseline within hours. However, the decrease in depressive symptoms occurred progressively across three days. The differing timelines suggested to the authors a disconnect between the euphoric and antidepressant effects of ketamine.

In 2006, Zarate et al. tested ketamine's ability to treat MDD in a trial of 18 treatment resistant patients — defined here as patients who did not respond to at least one

adequate trial of an antidepressant. They found ketamine, within hours of administration, had robust antidepressant activity in this population. For 71% of patients, symptoms decreased in severity by at least one half.

Regarding the mechanism of action, Zarate et al. (2006) argued that the delayed therapeutic effect of traditional monoaminergic drugs could be accounted for by the delayed effects of these monoaminergic drugs on downstream neurotrophic signaling cascades and glutamate transmission. The changes in these signaling cascades and the glutamate system then cause improvements in mood (Zarate et al., 2006).

Following these initial studies, research on ketamine and depression has multiplied. In the effort to understand the neurobiology of depression, Duman & Aghajanian (Duman & Aghajanian, 2012) recently characterized ketamine's rapid antidepressant effect as "arguably the most important discovery in half a century." In March 2019, the Food and Drug Administration approved an intranasal formulation of ketamine for treating depressed patients unresponsive to previous treatments (Farchione, 2019).

This thesis will outline three aspects of ketamine's use in the treatment of depression. First, ketamine's efficacy and clinical utility in the treatment of depression will be assessed. Second, the safety and tolerability of ketamine in this group of patients will be described. Third, hypothesized mechanisms of action for ketamine's antidepressant effect will be explored.

Specific Aims

The specific aims of this thesis are:

1. Evaluation of the effectiveness and clinical utility of ketamine in the treatment of depression.
2. Examination of ketamine's safety and tolerability in depressed patients.
3. Description of proposed mechanisms of action underlying ketamine's antidepressant effect.

EFFICACY AND CLINICAL UTILITY

Ketamine alleviates depression within hours and patients often benefit from the effects for up to one week after the infusion. The drug is more effective than placebo in both unipolar and bipolar depression, but has the greater effect in unipolar depression. An intravenous ketamine dose of 0.5 mg/kg infused within 40 minutes has been found to maximize improvements in mood while avoiding the greater side effects caused by higher doses.

Combination treatments and non-intravenous routes of administration have been tested in an effort to broaden ketamine's clinical utility. Repeated ketamine infusions may potentially extend antidepressant effects beyond what is typically seen with a single infusion; in contrast, prolonged ketamine infusions do not appear to extend the duration of action. Combining ketamine with escitalopram or CBT may enhance and prolong antidepressant effects. Treating patients with D-cycloserine following ketamine infusion does not prolong antidepressant effects beyond the single week in which ketamine typically acts. Besides intravenous infusion, ketamine has also shown antidepressant effects when administered by oral, sublingual, and nasal routes.

Ketamine may also be useful in preventing suicide. Within one day, ketamine decreases suicidal ideation. Upwards of 25% of patients are free of suicidal ideation for a week. The drug has the potential to be developed as an alternative to ECT in the treatment of suicidality.

Alongside evidence in favor of ketamine's utility as an antidepressant and anti-suicide agent, concerns exist about ketamine's effectiveness. Doubt about the integrity of blinding procedures burdens many studies. Some researchers and clinicians also worry that ketamine is a euphoriant rather than an antidepressant.

Remission and Response Rates

Ketamine infusion produces an anti-depressant effect within two hours. The effects are significant when measured with the Beck Depression Inventory, the Montgomery-Åsberg Depression Rating Scale (MADRS), the HDRS, and other measures of depression. The impact is greatest on the day of the infusion and antidepressant effects are sustained for about a week. No other treatment is known to combine such rapidity of action with a relatively sustained effect (Wilkinson, Ballard, et al., 2017; Zarate et al., 2006).

A meta-analysis composed of seven randomized control trials (RCTs) found ketamine to have a nearly 10 fold higher likelihood of inducing a response one day after treatment in comparison to control (Newport et al., 2015). Response is defined here and throughout this thesis as a 50% reduction of depressive symptoms from baseline, unless otherwise noted. One day following treatment, 53% of patients achieved response.

The same meta-analysis found ketamine, one day following treatment, to have a 14 fold higher likelihood of inducing remission of depressive symptoms versus control (Newport et al., 2015). Remission of depression is defined as having minimal to no symptoms and a return to normal function. However, no standardized exact criteria for

remission exists and the criteria can vary across studies (Israel, 2006), as was the case in this meta-analysis (Newport et al., 2015). The meta-analysis showed that 30% of patients achieved remission one day after treatment.

In the Newport et al. (2015) meta-analysis, response and remission rates declined one week after ketamine treatment, but a clinically meaningful antidepressant effect persisted for a subset of patients. Response was retained by 31% of patients and remission by 15%.

Unipolar Versus Bipolar Depression

A meta-analysis of eight RCTs (McGirr et al., 2015) found ketamine to be more effective than placebo in treating both unipolar depression and bipolar depression. However, ketamine appeared to have a greater antidepressant effect in unipolar depression. The effect size for unipolar patients was a standardized mean difference of 1.07 between ketamine and control; for bipolar patients, the standardized mean difference was 0.68. Additionally, ketamine did not seem to produce mania or psychotic symptoms in the bipolar patients, although the number of patients with bipolar depression in the sample was small. Of the 34 patients with bipolar depression, the only patient to experience mania was receiving saline control.

Dosing

Almost all ketamine trials for depression have used a parenteral dose of 0.5 mg/kg infused over 40 minutes (Murrough et al., 2017). A one-month National Institute of Mental Health RCT of 99 patients examined four intravenous doses of ketamine. Doses of 0.5 mg/kg and 1 mg/kg alleviated depression significantly more than the midazolam control. Doses of 0.1 mg/kg and 0.2 mg/kg were not superior to control. Doses of 0.5 mg/kg and 1 mg/kg did not show a statistically significant difference in antidepressant effects, but the 0.5 mg/kg dose actually produced the greater numerical decrease in depression scores. Compared to the 0.5 mg/kg dose, the 1 mg/kg dose caused greater percentages of patients to experience adverse events — including dissociative and elevated blood pressure side effects (Fava et al., 2018).

Repeated and Prolonged Ketamine Infusions

While an attenuation of symptoms for one week is meaningful, a key concern is finding a way to maintain ketamine's anti-depressant effect. Researchers have attempted to extend ketamine's duration of action by administering repeated ketamine infusions and prolonged ketamine infusions.

An open-label study of 24 patients with treatment resistant depression examined the effect of repeated ketamine infusions. Treatment resistant depression was defined in this study as a depressive episode that has resisted at least two Food and Drug Administration approved antidepressant treatments. Six ketamine infusions across a 12-

day period produced a mean time to relapse among ketamine responders of 18 days after the last infusion. Subjects displayed significant variability with some maintaining response for more than 83 days (Murrough et al., 2013). The best dosing frequency for repeated infusions (daily vs. more intermittently) is still currently unknown (Murrough et al., 2017).

A second study (Lenze et al., 2016) compared the effects of a 96-hour ketamine infusion to a 40-minute ketamine infusion. Clonidine was administered to attenuate the psychotomimetic and sympathomimetic side effects produced by the infusion. Though not formally tested, the clonidine did seem to prevent most psychotomimetic side effects and limit blood pressure elevations to clinically insignificant levels. However, no significant difference was found between the 96-hour and 40-minute groups in the time that ketamine's antidepressant effect was maintained after the infusion. The sample size in this study (10 subjects) limited statistical power. These two studies (Lenze et al., 2016; Murrough et al., 2013) illustrate the significant challenges that remain in extending ketamine's duration of action.

Combination Treatments

Ketamine has been combined with other treatments to accelerate, enhance, or extend ketamine's therapeutic effect. The selective serotonin reuptake inhibitor escitalopram has been given along with ketamine to achieve antidepressant response and remission more quickly and extend antidepressant effects beyond what is usually achieved with a single ketamine infusion. Following ketamine infusions, D-cycloserine

has also been given to patients to try to enhance and extend ketamine's antidepressant effect. Lastly, CBT has been used to try to extend the antidepressant effect attained through ketamine infusion.

An RCT (Y.-D. Hu et al., 2016) examining 30 outpatients with MDD found that augmenting escitalopram treatment with a single ketamine administration quickened the onset of antidepressant effects in comparison to escitalopram treatment alone. In this study remission was defined as a MADRS score of less than 10. Such a MADRS score corresponds with recovery or only mild symptoms (Müller, Szegedi, Wetzel, & Benkert, 2000). Four weeks after receiving ketamine or saline and beginning escitalopram treatment, patients in the ketamine group had higher response and remission rates in comparison to the saline group. The mean time to reach response was shorter with ketamine: six days for the ketamine group versus 27 days for the saline group. More patients did experience at least one adverse event in the ketamine group, but no patients discontinued treatment as a consequence (Y.-D. Hu et al., 2016).

D-cycloserine is an antituberculosis drug that shows antidepressant effects after eight weeks of treatment. Like ketamine, D-cycloserine inhibits NMDARs. Seven subjects received open-label ketamine, followed by eight weeks of D-cycloserine. An improvement after ketamine predicted improvement with D-cycloserine. However, a partial relapse at two weeks indicated that ketamine and D-cycloserine exerted antidepressant effects independently during the first two weeks and after eight weeks, respectively. In other words, although D-cycloserine may possess its own antidepressant effects, there was no indication that D-cycloserine extended ketamine's antidepressant

effect or acted synergistically with ketamine to enhance ketamine's antidepressant effects (Kantrowitz, Halberstam, & Gangwisch, 2015).

One study has shown CBT augmenting ketamine's effect over a longer time period compared to ketamine monotherapy. In the open-label study, four ketamine treatments were given over two weeks. Concurrently, the patients received 10 weeks of CBT. Among the patients that responded to ketamine, 75% had not relapsed eight weeks after the final ketamine exposure. The median time to relapse for ketamine responders was 12 weeks with CBT. The result compares favorably with similar open-label ketamine monotherapy studies, in which the number of responders who had not relapsed at only four weeks ranged from 89 – 55%. In these ketamine monotherapy studies without CBT, median time to relapse was about three weeks (ranging from 17 – 25 days). However, the small sample size and lack of a control group in this study make these results preliminary (Wilkinson, Wright, et al., 2017).

Other Routes of Administration

A current limitation for ketamine in the treatment of depression is that the drug must be administered intravenously, limiting its use in outpatient settings (Daly et al., 2018). In the first paper (Berman et al., 2000) to examine ketamine's antidepressant effect in humans, the authors speculated that the intravenous route of administration might at least partially account for ketamine's rapid effect. They note that some studies of intravenously administered tricyclic antidepressants show similarly rapid antidepressant effects. Subsequent studies of ketamine administered orally, sublingually, and nasally

have shown roughly comparably antidepressant effects to ketamine administered intravenously, though oral administration does not achieve an antidepressant effect as rapidly.

An open-label study of oral ketamine (Irwin et al., 2013) gave patients with depression a daily oral dose of 0.5 mg/kg ketamine for 28 days. Response rates in this study — assessed here by the Hospital Anxiety and Depression Scale — were similar to those for intravenous ketamine. However, this study defined response atypically as a 30% or more decrease in depression scores. Additionally, antidepressant effects took longer to take hold: in comparison to before treatment, the decrease in depression did not become significant until 14 days after treatment. The antidepressant effect remained significant compared to baseline until the end of the study at day 28. Anxiolytic effects became significant on the third day. Few adverse events were recorded. One subject each reported mild complaints of diarrhea, insomnia, and akathisia.

An open-label study of sublingual administration (Lara, Bisol, & Munari, 2013) found a comparable response rate (77%) to intravenous studies. However, the improvement necessary for a response was not defined in this study. No standardized diagnostic scales were used; instead, clinicians assigned response and remission designations to patients according to the reports of patients and relatives. Unipolar or bipolar depression patients sublingually absorbed ketamine doses of 10 mg drawn from a 100 mg/ml solution for five minutes and then swallowed the dose. The 26 subjects in the study received doses every two to three days or on a weekly basis. Side effects were mild.

A lightheadedness that subsided within 30 minutes of the dose was commonly experienced.

Esketamine, the S-enantiomer of ketamine, has been developed as an intranasal formulation. In a 2017 RCT sponsored by Janssen Research & Development that evaluated the intranasal formulation (Daly et al., 2018), the participants had failed one to three previous antidepressants. Patients received placebo, 28 mg, 56 mg, or 84 mg esketamine twice weekly for two weeks. After that, in a nine-week open label phase, all patients received esketamine twice weekly for the first two weeks, weekly for the next three weeks, and then once every two weeks for the remaining four weeks. Lastly, patients were followed another eight weeks in a follow-up phase without any further administration of ketamine.

At the 24-hour and one-week time points following treatment, there was a statistically significant decrease in MADRS scores in all esketamine groups compared to placebo. There was some further decrease in MADRS scores with repeated dosing, as seen in the open label phase. Notably, in the eight-week follow-up phase, the decrease in MADRS scores was maintained for the majority of participants without any further ketamine doses during those eight weeks. With the lowest dose (28 mg), the duration of efficacy beyond the treatment period seemed to be shorter and fewer patients achieved a 50% reduction in symptoms.

Use for Suicide Prevention

Does ketamine diminish suicidal ideation more quickly than control? Is the anti-suicide effect independent of ketamine's diminution of other symptoms of depression? Wilkinson et al., 2017 used systemic review and meta-analysis to examine ketamine's effectiveness as an anti-suicide agent. Only single dose, comparison intervention trials using saline and midazolam as controls were included. Wilkinson et al., 2017 identified 11 relevant trials, encompassing 167 patients who presented with suicidal ideation.

The meta-analysis found that ketamine diminished suicidal ideation more quickly than control treatments as demonstrated by two clinician rating scales: the MADRS and HDRS. Ketamine also significantly increased the number of patients free from suicidal ideation as assessed by these clinician-administered rating scales. A substantial effect was observed as early as day one and the effect extended for up to a week. Effect sizes were large to moderate ($d = 0.85$ on day one and $d = 0.61$ on day seven). Self-report measures of suicidal ideation — namely, items of the Quick Inventory of Depressive Symptomatology–Self Report (QIDS-SR) and the Beck Depression Inventory — showed a similarly significant reduction after ketamine infusion.

Ketamine's reduction of suicidal ideation occurred equally across age, gender, race, concomitant use of psychotropics, and outpatient versus inpatient status covariates. Over half of participants reported no suicidal ideation for one week after infusion. For one patient to be free of all suicidal ideation across the entire first week post-infusion, the number needed to treat was 3.1 – 4 patients.

Reduction of depressive symptoms and suicidal ideation were highly correlated across the first week after infusion. After controlling for the decrease in depression symptoms, ketamine's reduction of suicidal ideation continued to be significant. This indicates ketamine had a specific effect on suicidal ideation beyond its more general antidepressant effects.

ECT is the standard of care for patients with acute suicidal ideation and a mood disorder. Wilkinson, Ballard, et al. (2017) performed a comparison between ketamine infusion results from their meta-analysis and an open-label ECT trial. Within 24 hours of a single ketamine infusion, 55% of patients were free of suicidal ideation. One week post-infusion, 60% were free of suicidal ideation. In contrast, 38% of patients were free of suicidal ideation after three ECT treatments in a single week. Within a one-week timespan then, ketamine is potentially a superior treatment.

However, over a longer period of time, the available evidence favors the use of ECT. After six ECT treatments (occurring over two weeks following the start of treatment), 61% of patients were free of suicidal ideation. At the end of the acute course of ECT treatment (on average 7.5 treatments with ECT administered on a thrice weekly schedule), 81% of patients were free of suicidal ideation. The ketamine meta-analysis did not include data beyond one week so a direct comparison with ECT cannot yet be made beyond one week (C. H. Kellner et al., 2005; Wilkinson, Ballard, et al., 2017).

Repeated ketamine infusions, however, could have longer lasting anti-suicide effects beyond acute treatment, unlike repeated ECT. After acute ECT treatment, relapse rates for depression and suicidal ideation are substantial if further treatment is not

sustained (C. H. Kellner et al., 2005; J. Prudic & Sackeim, 1999). For example, in an observational study of 154 patients who were followed after remission with ECT treatment, 64% of patients relapsed within six months (Joan Prudic, Olfson, Marcus, Fuller, & Sackeim, 2004). Remission after ECT treatment was defined in that study as at least a 60% reduction in HDRS score and an HDRS score of less than 10. In comparison, the eight week follow-up phase of the intranasal study of ketamine (Daly et al., 2018) indicates the potential for sustained resolution of depressive symptoms with repeated ketamine treatments. If suicidal ideation is assumed to have decreased along with depression, repeated intranasal ketamine has the potential to treat suicidal ideation for weeks after the treatment ends. However, the intranasal study of ketamine excluded patients with any current or recent suicidal ideation. As such, the effectiveness of repeated ketamine treatments as an anti-suicide agent beyond acute treatment is currently unproven (Daly et al., 2018).

Ketamine also potentially has a more favorable side effect profile than ECT, although ketamine's long-term side effects have not yet been rigorously studied (see Safety and Tolerability section below). Medical adverse effects caused by ECT include cardiopulmonary events, fractures, dental injuries, and tongue wounds. Cognitive adverse effects caused by ECT include confusion, retrograde amnesia, and anterograde amnesia (C. Kellner, 2018). Rates of persistent memory loss after ECT ranged from 29% to 55% (Rose, Fleischmann, Wykes, Leese, & Bindman, 2003).

Most of the patients in the meta-analysis evaluating ketamine's effectiveness against suicide (Wilkinson, Ballard, et al., 2017) were patients who had MDD or bipolar

depression. Future studies might examine ketamine's effectiveness as an anti-suicide agent across a wider range of diagnoses. In particular, patients with a substance use disorder or schizophrenia/schizoaffective disorder were excluded from the populations studied in the meta-analysis.

A second limitation of the included studies is that they only assess suicidal ideation rather than suicidal behavior. Only one of the 11 studies included patients believed to be at immediate risk of suicide. Additionally, patient outcomes were not monitored beyond one week after the ketamine infusion (Wilkinson, Ballard, et al., 2017).

Concerns About Efficacy

Among the studies evaluating ketamine's effectiveness as an antidepressant and anti-suicide agent, a concern recurs about the integrity of the blinding method (Murrough et al., 2017). For example, in Berman et al. (2000) and Fava et al. (2018) patients could readily discern whether they received ketamine or saline. Studies that used saline as a comparator had larger effect sizes than those that used midazolam (Wilkinson, Ballard, et al., 2017). Unblinding may occur in studies that use midazolam as well, as occurred in Fava et al. (2018).

A second concern centers on whether ketamine is a bona fide antidepressant or is simply a euphoriant (Schatzberg, 2014). However, as noted in Berman et al. (2000), the timeline of events following ketamine administration does seem to distinguish between euphoric and antidepressant effects. Zarate et al. (2006) demonstrated that ketamine's

euphoric effects returned to baseline within 110 minutes of infusion for all patients, as assessed by the Young Mania Rating Scale. In contrast, ketamine's antidepressant effect lasts for approximately one week (Zarate et al., 2006).

SAFETY AND TOLERABILITY

Ketamine is considered safe as an anesthetic. However, as an anesthetic, ketamine is usually only given to a patient as a one-time dose (Short, Fong, Galvez, Shelker, & Loo, 2018). Such single doses of ketamine generally only produce acute side effects that resolve within hours of the administration. In contrast, less is known about the side effects that may result following multiple ketamine administrations. With repeated ketamine doses, concerns exist about genitourinary, hepatic, and cognitive adverse effects as well as the risk of dependency. A small number of studies on other routes of ketamine administration aside from the most commonly used intravenous route (e.g., intranasal) have been performed. In comparison to the side effect profile of intravenous administration, these other routes show a largely similar mix of side effects.

Single Doses

In treating depression, ketamine is generally safe and well-tolerated when used under supervision in single doses. Ketamine infusions can induce perceptual disturbances, confusion, elevated blood pressure, euphoria, and dizziness among other side effects. However, these adverse events mostly dissipate within 80 minutes of the infusion (Zarate et al., 2006). Other side effects commonly reported are blurred vision and nausea (Short et al., 2018).

A meta-analysis of 60 studies (Short et al., 2018) found that the most common psychiatric short-term side effect was anxiety. The anxiety was generally acute and self-

limited. Other psychiatric side effects included agitation, irritability, euphoria, delusions, unusual thoughts, apathy, and panic. These side effects, too, tended to be acute and self-limiting.

The same meta-analysis (Short et al., 2018) found that the most common psychotomimetic side effect was dissociation. Other psychotomimetic side effects include perceptual disturbances, odd or abnormal sensations, derealization, hallucinations, feeling strange or unreal, and depersonalization. These side effects also dissipate within a few hours of the infusion. No long-term psychotomimetic side effects have been observed.

In Short et al. (2018), the most common cardiovascular changes were increased blood pressure and heart rate. In general, these cardiovascular changes returned to baseline within 90 minutes (Short et al., 2018). The mechanism causing the transient increase in blood pressure is thought to be an increase in cardiac output (Daly et al., 2018).

Cognitive side effects encompass poor memory, decreased concentration, confusion, and diminished mental capacity. The available studies have largely documented these acute effects only occurring within four hours of the ketamine infusion. However, most studies did not assess cognitive side effects (Short et al., 2018). An RCT (Murrough et al., 2015) found ketamine administration to have no difference from midazolam in terms of cognitive effects one week following administration. In that study, neurocognitive performance was measured before drug administration and seven days after. Performance actually improved in both the ketamine and midazolam groups at seven days, likely as the result of a general learning effect.

Repeated Doses

There is less data available on the safety of multiple doses of ketamine (Short et al., 2018). The acute side effects mentioned above — most prominently dissociative effects and elevations in blood pressure — do occur with repeated infusions, but remain mild and do not increase in severity (Murrough et al., 2013). In evaluating ketamine's acute side effects (and also effectiveness), RCTs are useful. However, these trials tend to last a short period of time and use a limited number of doses and participants (Short et al., 2018). The meta-analysis of 60 studies examining side effects of ketamine administration (Short et al., 2018) found that long-term side effects were only examined in 20% of studies. In consequence, most of the available literature is not well suited to ascertaining long-term side effects or rare adverse events.

Individuals who have used ketamine over a longer period of time — namely, individuals with chronic pain and recreational users — show a number of adverse outcomes. Urinary tract symptoms and liver toxicity has been documented in these populations. Chronic recreational users of ketamine also show cognitive impairments (Short et al., 2018). Additionally, ketamine does have addictive properties. The risk of dependency constrains the drug's widespread use (Morgan & Curran, 2012; Zanos et al., 2016).

Short et al. (2018) report several genitourinary symptoms in chronic ketamine users, including cystitis, bladder dysfunction, an increase in urinary frequency and urgency, urge incontinence, and rarely hematuria with pain. In severe cases, renal damage has also been documented. The mechanism underlying ketamine's urological symptoms

are unknown. An in vitro study has shown that ketamine causes urothelial cells to apoptose. It is unclear if such damage to the bladder urothelium is reversed after ceasing ketamine use. Out of 60 studies in this review, urinary side effects were only assessed in five studies. The Independent Scientific Committee on Drugs (Morgan & Curran, 2012) determined that daily ketamine use is associated with ulcerative cystitis.

Hepatotoxicity has been reported following repeated or prolonged ketamine infusions. The mechanism underlying liver toxicity in ketamine users is also unknown. Enhanced free radical formation and lipid peroxidation, increased flow resistance across the Sphincter of Oddi, or dilatation of the biliary tree and gallbladder dyskinesia may play a role in this toxicity. Liver function tests were reported in seven of 60 studies. Two of these studies showed liver abnormalities following ketamine administration (Short et al., 2018).

Compared to healthy controls without a history of drug addiction, frequent recreational ketamine users can show substantial impairments of short and long-term memory. Chronic ketamine users exhibit an upregulation of dopamine receptor D1 availability in the dorsolateral PFC. The same effect is seen in animals subject to chronic dopamine depletion. Disruption of dopaminergic transmission by ketamine may mediate disruption of working memory and executive control (Short et al., 2018). Daily ketamine use is also associated with working and episodic memory impairments according to Morgan and Curran (2012).

However, the presence of cognitive deficits in non-recreational repeat users of ketamine — namely, depression and chronic pain patients — is less certain. For example,

a study of chronic pain patients given a five-day anesthetic dose of ketamine did not discover any cognitive deficits six weeks later (Koffler et al., 2007). Longer follow-up periods in patients with depression or pain using repeated ketamine have not yet been used to examine the presence of persistent cognitive deficits (Short et al., 2018).

In the context of frequent recreational use of ketamine, reports of ketamine craving, compulsive behavior, and tolerance are commonly reported. Pigeons and monkeys self-administer rising doses of ketamine over time (Short et al., 2018). Recreational users of ketamine frequently also self-administer increasing doses over time. A ketamine withdrawal syndrome may exist, but such a syndrome has not been well-studied or characterized (Morgan & Curran, 2012). Documented ketamine dependence has rarely been seen. In the past 20 years, fewer than 15 such cases have been reported (Short et al., 2018). However, no large studies have attempted to determine the incidence rate of ketamine dependency. In consequence, the risk of dependency remains difficult to quantify (Morgan & Curran, 2012).

Other Routes of Administration

Patients receiving intranasal esketamine experienced a similar side effect profile to those receiving intravenous ketamine. Transient elevations in blood pressure, dissociative effects, and perceptual symptoms were common in patients, but resolved within two hours in most cases. Dissociative and perceptual symptoms tended to decrease with repeated dosing (Daly et al., 2018). Studies using oral, subcutaneous, or

intramuscular routes of administration tend to record fewer side effects than studies using intravenous routes (Short et al., 2018).

MECHANISM OF ACTION

Ketamine's mechanism of action is likely to be complex. The drug has multiple effects on glutamatergic transmission. Ketamine inhibits GABAergic interneurons — increasing glutamate release — and activates AMPARs. Downstream effects on intracellular mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase (ERK) signaling may be critical to ketamine's effects. Ketamine also inhibits glycogen synthase kinase 3 (GSK3) and blocks apoptotic extrasynaptic NMDARs. All these effects of ketamine are predicted to increase BDNF production, but tests of BNDF levels in ketamine treated patients display mixed findings.

Studies comparing ketamine to other NMDAR antagonists and enhancers indicate that ketamine's antagonism of NMDARs alone might not account for its antidepressant effect. Instead, multiple actions by ketamine on the glutamate system converge to produce synaptogenesis, which might be the key mechanism in alleviating depression.

Researchers have also parsed the differential effects of ketamine's two stereoisomers and delineated ketamine's varied impact across a number of brain regions. The two stereoisomers of ketamine, *(R)*- and *(S)*-ketamine, seem to have differing mechanisms of action, efficacy, and side effect profiles. Researchers have used brain imaging to show how ketamine alters regional neural activity and connectivity. Ketamine increases activity and connectivity across some regions while decreasing them across others.

Inhibition of GABAergic Interneurons

Initially, ketamine's mechanism of antidepressant action was thought to be inhibition of NMDARs. Ketamine binds multiple targets, but binds NMDARs with the highest affinity (Zarate et al., 2006). In particular, ketamine binds to a site within the NMDAR ion channel, acting as a non-competitive antagonist (Murrough et al., 2017). These receptors mediate ketamine's anesthetic effects (Zanos et al., 2016).

The initial hypothesis of multiple research groups was that ketamine inhibited NMDARs on GABA-releasing interneurons (**Figure 3**). These interneurons synapse onto prefrontal pyramidal glutamatergic neurons. Inhibition of the GABA releasing interneurons by ketamine was thought to disinhibit downstream prefrontal pyramidal glutamatergic signaling. These disinhibited pyramidal neurons then released a surge of glutamate. The resultant increase in glutamatergic transmission in mood associated brain regions was thought to be responsible for ketamine's antidepressant effect (Li et al., 2010; Murrough et al., 2017; Zanos et al., 2016).

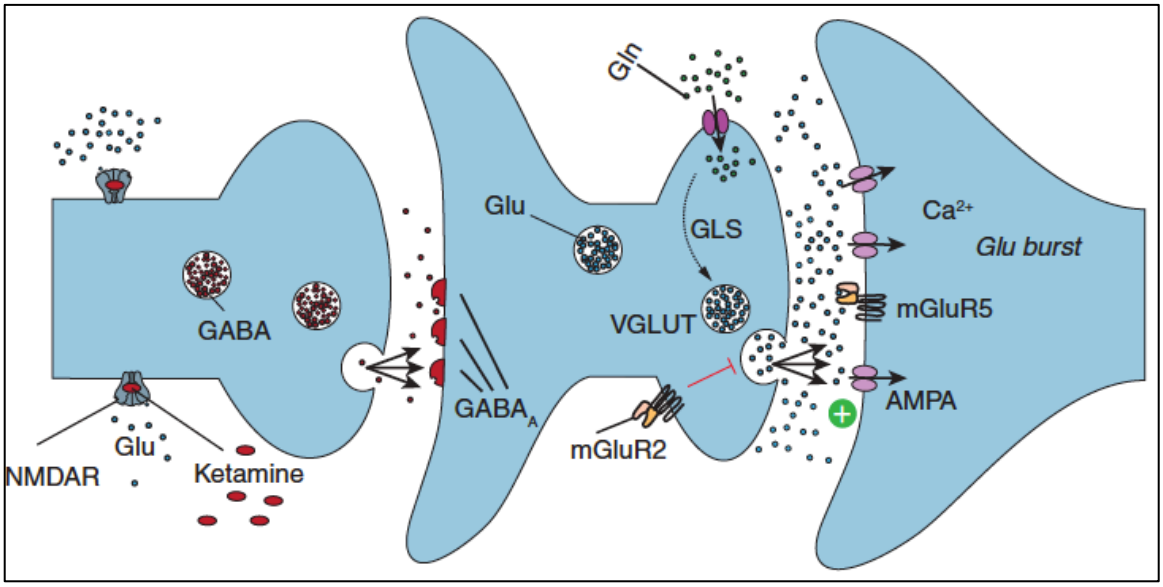

Figure 3: Ketamine Inhibiting NMDARs on GABAergic Neurons Causes a Burst of Glutamate Release. As shown on the leftmost neuron, ketamine binds pre-synaptic NMDARs, leading to decreased GABA release from these interneurons. With GABA release lowered, downstream glutamate exocytosis by prefrontal pyramidal neurons (middle neuron) is enhanced. This surge of glutamate activates AMPA receptors, as depicted in the rightmost neuron. Early research implicated this sequence of events as underlying ketamine's antidepressant effect. *AMPA*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; *GABA*, γ–aminobutyric acid; $GABA_A$, γ–aminobutyric acid A receptor; *Gln*, glutamine; *GLS*, glutaminase; *Glu*, glutamate; *mGluR*, metabotropic glutamate receptor; *NMDAR*, *N*-Methyl-D-aspartate receptor; *VGLUT*, vesicular glutamate transporter. Figure from (Kosten, Verhaeghe, wyffels, Stroobants, & Staelens, 2018).

Activation of Intrasynaptic AMPARs

Low dose ketamine also directly or indirectly activates intrasynapatic AMPARs. AMPAR activation is a prerequisite for intrasynpatic NMDAR activation. Ketamine may then indirectly enhance signaling at intrasynaptic NMDARs as a consequence of ketamine's activation of AMPARs. This activation of AMPARs may be key to ketamine's mechanism of action (Murrough et al., 2017).

A research group also recently discovered a ketamine metabolite that activates AMPARs (**Figure 4**). The ketamine metabolite (2R,6R)-hydroxynorketamine (HNK) was shown to induce antidepressant effects in animal models. Stopping metabolism of ketamine to HNK blocked ketamine's antidepressant effects in these same animal models. In rats, HNK does not inhibit NMDARs of interneurons; instead, HNK increases the frequency and amplitude of AMPAR mediated excitatory post-synaptic potentials (Zanos et al., 2016). Blockade of AMPARs abolishes HNK's antidepressant effect in mice. AMPAR blockade also reduces the antidepressant effect of other compounds, including the NMDAR partial antagonist GLYX-13; the mGluR2 and mGluR3 antagonist LY341495; and the muscarinic acetylcholine receptor antagonist scopolamine (Murrough et al., 2017).

The above research on ketamine's activation of AMPARs indicates that blockade of interneuron NMDARs may not be necessary for ketamine's antidepressant effect, as was originally believed (Zanos et al., 2016). In short, Zanos et al. (2016) argue that activation of AMPARs by HNK is necessary and sufficient for ketamine's antidepressant effects in animal models.

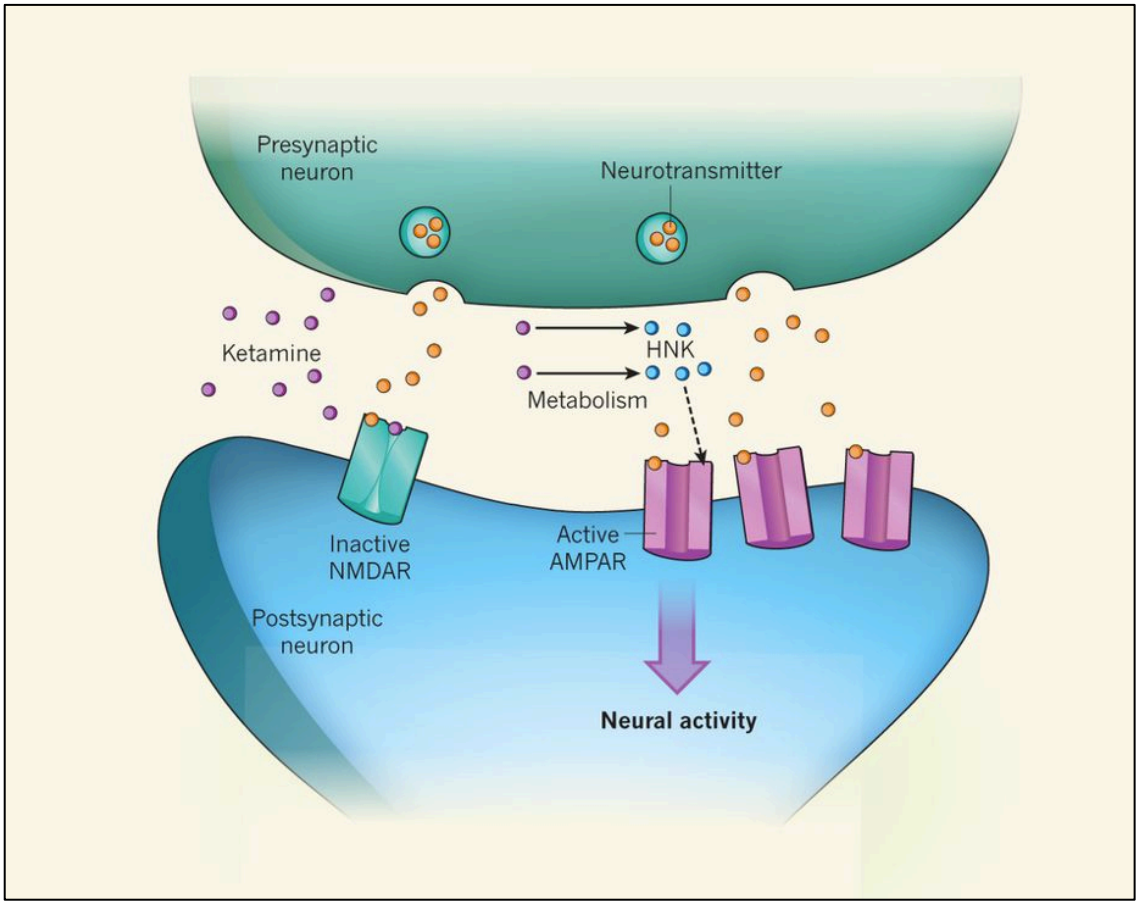

Figure 4: Ketamine Metabolite HNK Activates AMPARs. Ketamine is metabolized into HNK, which is thought to directly activate intrasynaptic AMPARs. AMPAR activation then assists in the activation of intrasynaptic NMDARs. In animal models, halting metabolism of ketamine into HNK or blocking AMPARs eliminates ketamine's antidepressant effect. *AMPAR*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; *HNK*, (2R,6R)-hydroxynorketamine; *NMDAR*, *N*-Methyl-D-aspartate receptor. Figure from (Malinow, 2016).

BDNF, ERK–Akt, and mTORC1 Signaling Enhance Synaptic Plasticity

Ketamine quickly alters intracellular signaling pathways of neurons in the PFCs of rats (Li et al., 2010). By activating AMPARs and intrasynaptic NMDARs, ketamine increases cytosolic Ca^{2+} concentration within neurons (**Figure 5**). The rise in Ca^{2+}

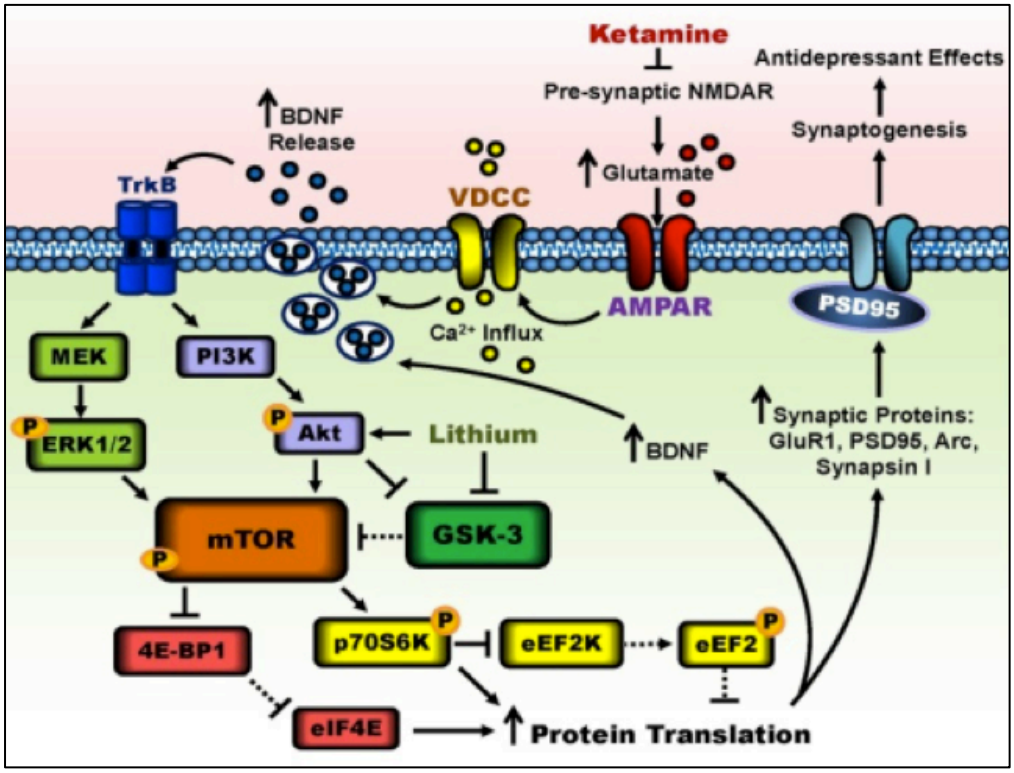

Figure 5: Ketamine Increases BDNF, ERK-Akt, and mTOR Signaling While Inhibiting GSK3. Ketamine inhibits pre-synaptic NMDARs of GABAergic neurons. The decrease in GABA disinhibits glutamate release. Elevated glutamate activates AMPARs, which may also be directly activated by ketamine or its metabolite HNK. AMPAR activation triggers Ca^{2+} influx through other channels, followed by exocytosis of the neurotrophin BDNF. BDNF binds TRKB receptors, activating ERK, Akt, and mTOR signaling. Translation of synaptic proteins, as well as of BDNF itself (not shown), results because of the increase in mTOR activity. Increased amounts of these proteins enhance synapse formation and strength, causing antidepressant effects. Additionally, ketamine — like lithium — inhibits GSK3 (not shown). Inhibition of GSK3 also increases mTOR activity and eventually synaptogenesis. *4E-BP1*, eukaryotic translation initiation factor 4E-binding protein 1; *Akt*, Protein Kinase B; *AMPAR*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; *Arc*, activity-regulated cytoskeleton-associated protein; *BDNF*, brain-derived neurotrophic factor; *eEF2*, eukaryotic elongation factor 2; *eEF2K*, eukaryotic elongation factor 2 kinase; *eIF4E*, eukaryotic translation initiation factor 4E; *ERK*, extracellular signal-regulated kinase; *GABA*, γ–aminobutyric acid; *GluR1*, glutamate receptor 1 subunit; *GSK3*, glycogen synthase kinase 3; *HNK*, (2R,6R)-hydroxynorketamine; *MEK*, mitogen-activated protein kinase kinase; *mTOR*, mammalian target of rapamycin; *NMDAR*, *N*-Methyl-D-aspartate receptor; *p70S6K*, ribosomal protein S6 kinase beta-1; *PI3K*, phosphoinositide 3-kinase; *PSD95*, post-synaptic density protein 95; *TRKB*, tropomyosin-related kinase B; *VDCC*, voltage-dependent calcium channels. Figure from (Scheuing, Chiu, Liao, & Chuang, 2015).

concentration triggers exocytosis of BDNF. BDNF binds tropomyosin-related kinase B (TRKB) receptors on neurons. TRKB receptor activation then enhances ERK–Akt and mammalian target of rapamycin complex 1 (mTORC1) signaling (Murrough et al., 2017). Activation of mTOR is dose dependent, occurring with the lower doses of ketamine used for depression. The higher ketamine doses used in anesthesia do not activate mTOR (Li et al., 2010). Increased signaling in ERK-Akt and mTORC1 pathways, in turn, enhances the translation of several proteins. These proteins include GluR1 (also called GluA1), post-synaptic density protein 95 (PSD95), and BDNF itself (Murrough et al., 2017). The induction of mTOR also increases the amount of the presynaptic protein synapsin I (Li et al., 2010).

By elevating the levels of these proteins, ketamine enhances synapse formation. GluR1, PSD95, and synapsin I are important for the formation, maturation, and maintenance of synapses and new synaptic spines (Li et al., 2010). Synapsin I, in particular, is critical for presynaptic plasticity enhancement and long-term potentiation (Kushner et al., 2005). Levels of GluR1, PSD95, and synapsin I remain higher for up to 72 hours after ketamine administration (Li et al., 2010). Increased BDNF also enhances the formation and maturation of synapses (B. Hu, Nikolakopoulou, & Cohen-Cory, 2005). The effects peak at two to six hours after ketamine infusion. Within two hours, mTOR phosphorylation — which indicates active mTOR signaling — returns to baseline (Li et al., 2010). In summary, ketamine infusion — operating through induction of BDNF, ERK-Akt, and mTORC1 pathways — results in the production of proteins that enhance synaptogenesis.

Several lines of evidence support the link between ketamine, AMPAR activation, synaptogenesis, and antidepressant effects. Two-photon imaging and electrophysiological studies in rats have demonstrated ketamine's ability to induce the formation of new synapses and strengthen existing synapses. Blockade of the mTOR pathway in rat PFCs abolished ketamine's antidepressant action in animal models of depression. Additionally in rats, selective inhibition of AMPARs blocked phosphorylation-mediated activation of ERK, Akt, and mTOR and also abolished ketamine's antidepressant effect (Li et al., 2010).

Despite the research supporting this chain of events, not all efforts to replicate the links between ketamine administration, an increase in mTORC1 signaling, and synaptic plasticity have succeeded (Murrough et al., 2017). Rapamycin, as an inhibitor of the mTOR pathway, might be expected to block ketamine's antidepressant effects. However, Autry et al. (2011) found that rapamycin did not, in fact, block ketamine's antidepressant effects in mice models. Additionally, rapamycin alone has antidepressant effects in mice when give daily for four days, but not when given after a single injection (Cleary et al., 2008). As such, the role of mTOR signaling in ketamine's antidepressant effect still remains unclear at present (Chun Yang et al., 2018).

Inhibition of Glycogen Synthase Kinase 3 Enhances Synaptic Plasticity

Ketamine is also thought to enhance synaptic plasticity through its effect on GSK3. Ketamine — like lithium — inhibits GSK3. Activation of GSK3 downregulates synaptic plasticity pathways (**Figure 5**). In consequence, ketamine and lithium disinhibit

pro-plasticity pathways. In hippocampal neurons, ketamine's inhibition of GSK3 causes an increase in GluR1 trafficking to the cell membrane. The increase in membranal GluR1 enhances AMPAR signaling and long-term potentiation (Murrough et al., 2017).

Ketamine Inhibits Extrasynaptic NMDARs

Ketamine is believed to block extrasynaptic NMDARs and, in doing so, provide a neuroprotective effect that inhibits apoptosis. Activation of extrasynaptic NMDARs inhibits protein translational machinery, including mTORC1 and eukaryotic elongation factor 2 (eEF2). Ketamine's blockade of extrasynaptic NMDARs causes eEF2 to be disinhibited (**Figure 6**). For this reason, the inhibition of extrasynaptic NMDARs by ketamine upregulates translation. Enhanced translation of BNDF and synaptic proteins increases synapse formation as well as synaptic strength, spine diameter, and density (Murrough et al., 2017).

Chronic stress has been shown to decrease intrasynaptic glutamatergic signaling and increase extrasynaptic glutamatergic signaling within hippocampal and cortical glutamatergic synapses. These brain regions have then shown reduced BDNF signaling. Ketamine rapidly reverses the effects of chronic stress on BDNF levels in the hippocampus (Murrough et al., 2017).

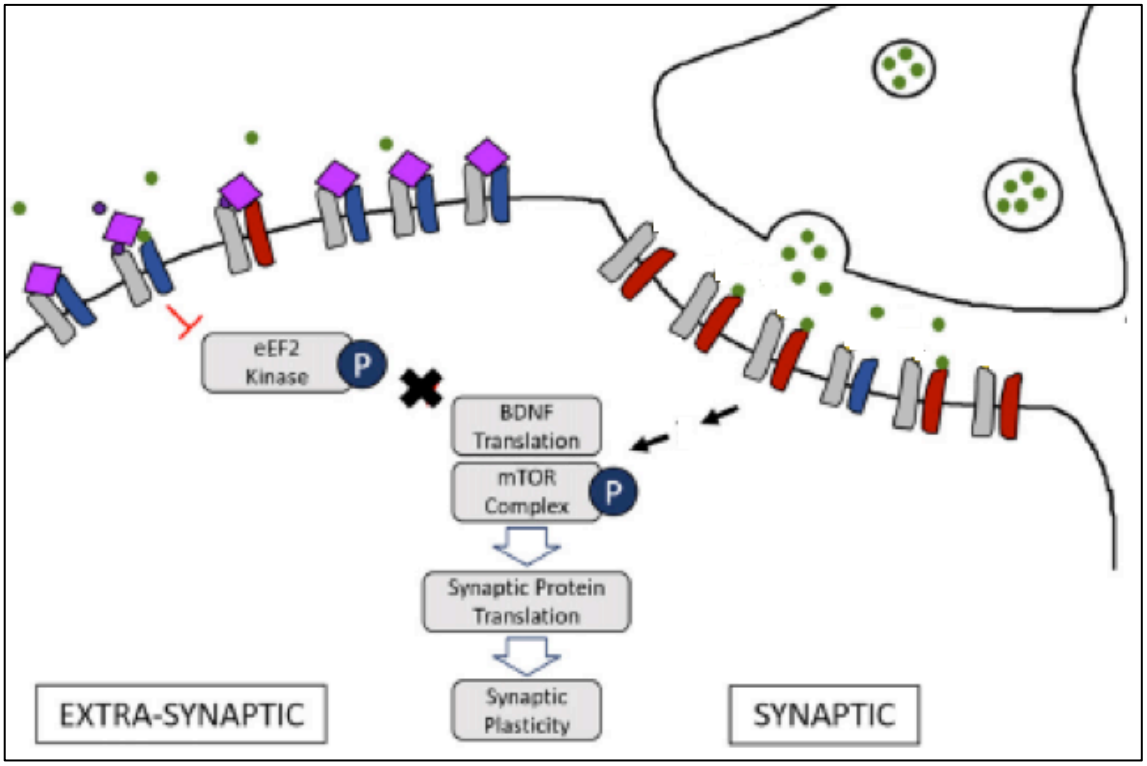

Figure 6: Activation of Intrasynaptic Versus Extrasynaptic NMDARs. When glutamate — shown as green dots — binds NMDARs within synapses, mTOR signaling is activated and synaptogenesis results. In contrast, the binding of glutamate to extrasynaptic NMDARs decreases synaptogenesis. Glutamate binding to extrasynaptic NMDARs causes a local influx of Ca^{2+}. The increased Ca^{2+} at this location activates eEF2 kinase, possibly through Ca^{2+} induced phosphorylation of eEF2 kinase at particular residues. Activation of eEF2 kinase causes phosphorylation of eEF2, inactivating eEF2 (not shown). Inactive eEF2 diminishes BDNF translation and synaptogenesis. However, ketamine — shown here as purple diamonds — blocks glutamate from binding to extrasynaptic NMDARs, preventing activation of eEF2 kinase and reversing this chain of events. In this fashion, ketamine allows eEF2 to remain active and enhances translation of synaptic proteins. *BDNF*, brain-derived neurotrophic factor; *eEF2*, eukaryotic elongation factor 2; *mTOR*, mammalian target of rapamycin; *NMDAR*, *N*-Methyl-D-aspartate receptor. Information on extrasynaptic NMDAR mediated activation of eEF2 kinase from (Autry et al., 2011). The effect of rising cytosolic Ca^{2+} on the phosphorylation and the activity of eEF2 kinase and eEF2 is from (Heise et al., 2014). A description of how Ca^{2+} influx at extrasynaptic versus synaptic locations can cause differences in cell activity can be found in (Hardingham & Bading, 2010). Figure adapted from (Chan, Matthews, & Burnet, 2017).

Predicted Increase in BDNF

Based on the downstream effects of ketamine's activation of AMPARs and blockade of extrasynaptic NMDARs (as outlined above), an increase in BDNF within the brain would be expected following ketamine administration. Studies evaluating this outcome are limited in that they have only been able to sample circulating levels of BDNF rather than BDNF in the central nervous system. Results have been mixed. One study showed an increase in circulating BDNF in those patients with treatment resistant depression who responded to ketamine (Murrough et al., 2017). In contrast, an earlier study in depressed patients demonstrated no increase in circulating BDNF following ketamine administration despite improvements in mood (Machado-Vieira et al., 2009).

Contrast Between Ketamine and Other NMDAR Agents

A number of other NMDAR modulating agents have been tested for antidepressant effects. Memantine is an NMDAR antagonist, but does not increase hippocampal BDNF translation and has no antidepressant effects. As such, NMDAR antagonism may not be sufficient for an antidepressant effect.

Though ketamine is an NMDAR antagonist, NMDAR enhancers have also been show to display anti-depressant effects. The NMDAR enhancers D-cycloserine, GLYX-13, NRX-1074, and sarcosine all show antidepressant effects in initial studies. These agents are believed to activate AMPARs and intrasynaptic NMDARs, leading to downstream increases in BDNF and synapotogenesis. That these contrasting agents also initiate an antidepressant effect suggests that a complex mechanism of action may

underlie ketamine's antidepressant capacity. Multiple modifications of glutamate signaling may be responsible for ketamine's antidepressant effect (Murrough et al., 2017).

Differential Effects of (*R*)- Versus (*S*)-Ketamine

Recent research has uncovered different efficacy and side effect profiles for ketamine's two stereoisomers. In animal models of depression, both *(R)*- and *(S)*-ketamine have antidepressant effects. However, *(R)*-ketamine's effects are stronger and last longer. Additionally, *(R)*-ketamine does not produce psychotomimetic side effects and appears to be less addictive than *(S)*-ketamine (Chun Yang et al., 2018).

(R)- and *(S)*-ketamine may operate through different intracellular pathways. In a study using the chronic social defeat model (Chun Yang et al., 2018), mice were exposed to an aggressor mouse for 10 minutes daily for 10 days. These stressed mice then received either *(R)*- or *(S)*-ketamine. Both enantiomers had antidepressant effects. Rapamycin and AZD8055 — both mTOR inhibitors — blocked the antidepressant effect only of *(S)*-ketamine. The ERK inhibitor SL327 blocked the antidepressant effect only of *(R)*-ketamine.

A discrepancy does exist regarding these results. Ketamine is normally administered as a racemic mixture. As a consequence, one might expect rapamycin to diminish ketamine's antidepressant effect only partially (Chun Yang et al., 2018). However, a previous study has found that rapamycin abolishes ketamine's antidepressant effect completely (Li et al., 2010). In contrast, another study found rapamycin to have no

effect on ketamine's antidepressant capacity (Autry et al., 2011). The reason for this discrepancy is unknown at this time (Chun Yang et al., 2018).

(*R*)-ketamine has fewer dissociative side effects than (*S*)-ketamine. (*S*)-$_{\text{ketamine}}$ has a 3 to 4 times higher affinity for NMDARs than (*R*)-ketamine. For that reason, (*S*)-ketamine is the more potent anesthetic and produces more psychotomimetic side effects (Chun Yang et al., 2018).

(R)-ketamine does not affect mTOR signaling, which may mean that *(R)*-ketamine is less likely to be addictive than racemic ketamine or *(S)*-ketamine. When habit-forming drugs are used, activation of mTOR signaling in the brain is associated with reward and excess drug seeking (Chun Yang et al., 2018). Results from mice studies using the conditioned place preference paradigm show behavioral differences in the effects of *(R)*-ketamine and *(S)*-ketamine, as described below (C. Yang et al., 2015).

Conditioned place preference occurs when animals prefer to spend time in a location because that place has been associated with rewarding events. The paradigm is used to measure the rewarding effects of addictive drugs (Huston, Silva, Topic, & Müller, 2013). *(S)*-ketamine increases the time mice spent in areas where they received the *(S)*-ketamine, whereas *(R)*-ketamine does not. These results indicate that *(R)*-ketamine may have fewer or none of the addictive properties associated with *(S)*-ketamine and racemic ketamine (C. Yang et al., 2015).

Regional Changes in Brain Activity and Connectivity

When examined with brain imaging, depressed patients show a number of abnormalities in regional brain activity and connectivity. Administration of ketamine results in changes in activity in some of these brain regions and in the links between regions. This section will assess changes in the lateral habenula (LHb), PFC, anterior cingulate cortex (ACC), and amygdala. Changes specific to bipolar depression will also be examined.

The LHb inhibits the reward centers of the brain, namely, the dopaminergic ventral tegmental area and the serotonergic dorsal raphe nuclei. The LHb primarily contains glutamatergic neurons. A number of studies show that hyperactivity of the LHb is associated with depression (Y. Yang et al., 2018).

Animal studies show that ketamine's antidepressant effect may occur in part through the LHb. In rats bred for congenital learned helplessness, local infusion of ketamine into the LHb produced rapid antidepressant effects. These antidepressant effects were demonstrated in both the forced swim test and the sucrose preference test (Y. Yang et al., 2018).

Burst firing of neurons in the LHb enhances network synchronization with other parts of the brain and may have a stronger inhibitory effect on downstream reward centers. The congenital learned helplessness rats showed increased burst firing in their LHbs in comparison to control rats. Optogenetic techniques demonstrated that increasing LHb burst firing in mice using photostimulation could drive depression-like phenotypes in the real-time place aversion assay and forced swim test (Y. Yang et al., 2018).

Ketamine was found to reduce burst firing — but not tonic firing — in the LHbs of mice. The drug is thought to reduce burst firing by blocking NMDARs. Other agents that block NMDARs also reduced burst firing, but agents that blocked AMPARs only reduced burst firing slightly. These results might in part explain how ketamine is able to produce an antidepressant effect within hours, but do not account for ketamine's longer acting antidepressant effects. As discussed above, increased BDNF and synaptogenesis might instead account for ketamine's longer acting antidepressant effects (Y. Yang et al., 2018).

The PFC is involved in working memory, self-regulation, and executive function (Akil et al., 2018). In patients with MDD, ketamine increases pooled ^{1}H-MRS measures of glutamate, glutamine, and GABA in the PFC within one hour of administration. The transient elevation of glutamate caused by ketamine administration is thought to enhance synaptic plasticity. Transient activation of synapses can upscale activity in a brain region and enhance synaptic strength in that area by increasing AMPAR insertion and synaptic density. These elevated glutamate levels are tied to ketamine's dissociative effects and a correlation has been found between the degree of dissociative effects and the strength of ketamine's antidepressant effect. This possibility seems to indicate that it may be difficult to separate ketamine's antidepressant effect from unwanted dissociative effects (Murrough et al., 2017).

In resting-state functional connectivity magnetic resonance imaging, global brain connectivity is a measure of the degree of correlation between the activity in any particular brain voxel and all the other voxels of the brain. Patients with depression have

reduced global brain connectivity between the PFC and the rest of the brain. They also have increased connectivity of the posterior cingulate, precuneus, lingual gyrus, and cerebellum with the rest of the brain. By 24 hours following ketamine administration, depressed patients showed an increase in PFC connectivity and a decrease in cerebellum connectivity. In effect, ketamine administration reverses the major global brain connectivity abnormalities seen in depressed patients (Abdallah et al., 2017).

Inducing sadness in healthy controls has been shown to increase activity in the subgenual ACC. Counter intuitively however, depressed patients show resting state hypometabolism in this brain area (Phan, Wager, Taylor, & Liberzon, 2002). In normal controls examined by functional magnetic resonance imaging, ketamine decreases the blood oxygen level-dependent (BOLD) signal in the subgenual ACC. In patients with MDD by contrast, ketamine increases the BOLD signal in the ACC. This increase was correlated with some measures of ketamine's antidepressant effect (Murrough et al., 2017).

The amygdala coordinates brain responses to affective stimuli. Depressed patients show higher activity in the amygdala when exposed to negative emotional stimuli. In normal controls, ketamine decreases the amygdala's response to negative affective stimuli, but not positive stimuli (Scheidegger et al., 2016). In MDD patients following ketamine administration, increased activation of the amygdala in response to happy faces and decreased activation in response to angry faces correlated with the degree to which the depression scores of these patients declined (Reed et al., 2018).

A positron emission tomography study of patients with bipolar depression (Nugent et al., 2014) examined changes occurring in response to ketamine. Decreases in depression were correlated with increases in the metabolism of the right ventral striatum, the subgenual ACC, and the dorsal cingulate cortex.

CONCLUSIONS AND FUTURE DIRECTIONS

Based on the available evidence, ketamine appears to be an effective, rapidly acting antidepressant treatment. A single administration remains effective for about a week. Ketamine treats both major depression and bipolar depression, though initial results show the drug is relatively more effective in major depression. Notably, ketamine shows efficacy in treatment resistant populations that have failed other antidepressant therapies. Future studies might further test ketamine's relative efficacy in bipolar depression and in other subtypes of depression, like post-partum depression or depression with anxious distress.

The conclusion that ketamine is effective for depression is weakened by the inadequacy of blinding procedures in the existing RCTs. Trials that have used midalozam as a control show a smaller antidepressant effect for ketamine compared to trials that used saline as a control. Even in trials that used midalozam as a control, subjects could correctly identify whether they received ketamine or not. The evidence in favor of ketamine's efficacy would be strengthened going forward by finding an active placebo control that kept patients and treatment providers blind to the treatment administered.

Following the initial work establishing ketamine's effectiveness, further research has tried to refine treatment protocols and enhance ketamine's effects. At this time, a dose of 0.5 mg/kg appears to be optimal. If non-intravenous routes of ketamine administration demonstrate efficacy and safety, ketamine could potentially be used as a more routine clinical agent. In this regard, the development of intranasal ketamine administration is a beneficial step forward. Prolonged ketamine infusions do not seem to

prolong the antidepressant effect. Repeated ketamine infusions or combination therapy with CBT may sustain ketamine's effect, but work in this area is still preliminary. If repeated ketamine infusions or combination treatments are found to create long lasting antidepressant effects, ketamine's clinical utility would increase considerably.

At present, ketamine is particularly promising in scenarios where rapidity of action is needed. These situations include adding ketamine to escitalopram treatment to accelerate the achievement of response and remission and also administering ketamine as an acute treatment for patients at risk of suicide. In treating suicidal ideation acutely, ketamine may possess a more favorable side effect profile and the potential for longer lasting beneficial effects with repeat infusions or combination treatments in comparison to the current standard of care, ECT. Further research establishing ketamine's utility as an add-on to conventional antidepressants and as an acute anti-suicide agent may be fruitful. At this time, significant unknowns remain, however, in whether ketamine's anti-suicide therapeutic effects can be prolonged.

Ketamine is largely safe when single doses are used under clinical supervision. Acute side effects — e.g., dissociation, elevation of blood pressure, and confusion — are common, but subside within a few hours of administration. Insufficient data exists to make conclusions about ketamine's long-term risk, particularly with repeated doses. Potential long-term risks include genitourinary damage, hepatotoxicity, cognitive deficits, and dependence. Most studies only monitored side effects in a passive fashion and only monitored side effects during the acute treatment period (Short et al., 2018). Uncertainty

about long-term side effects leaves the clinical utility of repeated ketamine doses in doubt at the present time.

In the future, studies would benefit from long-term monitoring for side effects and active, structured inquiry in regards to known or potential side effects (Short et al., 2018). In general, structured questioning increases the frequency of recorded drug side effects compared to unstructured interviewing (Naranjo, Busto, & Sellers, 1982). Collecting information on comorbidities, concomitant medications, and safety would also be useful in future studies. The differential side effect profile of ketamine enantiomers and metabolites is another area to be investigated in the future (Short et al., 2018).

Ketamine's mechanism of action is likely to be multi-pronged. On the molecular level, inhibition of GABAergic interneurons, enhanced AMPAR activation, increased mTOR and ERK-Akt signaling, blockade of extrasynaptic NMDARs, inhibition of GSK3, and increased BNDF production may all be involved and potentially interlinked. In the future, studies of compounds that manipulate particular aspects of this system — like inhibition of mTOR by rapamycin or enhancement of NMDAR signaling by sarcosine — may continue to help isolate the contribution or non-contribution of these mechanisms to ketamine's antidepressant effect.

Brain imaging shows changed activity and connectivity across particular brain regions following ketamine administration. Decreased burst firing in the LHb, increased activity in the PFC and subgenual ACC, and altered response in the amygdala to emotionally salient stimuli may be integral to ketamine's mechanism of action.

There may be an underlying difference in the mechanisms that constitute ketamine's antidepressant effect within the first hours of administration and those that sustain the effect over the following days. For example, direct infusion of ketamine into the LHb produces an antidepressant effect in mice within an hour of infusion, but it remains unclear if decreased burst firing in the LHb has a role in ketamine's antidepressant effect over the following days (Y. Yang et al., 2018). Future research may refine understanding of potential mechanisms of action by distinguishing between those that operate within hours of ketamine administration and those that occur subsequently. Future research could also focus on isolating the actions of *(R)*- and *(S)*-ketamine, as well as the actions of individual ketamine metabolites. The potentially differing mechanisms of these molecules may cause differences in antidepressant efficacy and side effects.

Though this thesis has focused on the glutamate system in attempting to understand ketamine's mechanism of action, other systems may be involved. Research is being conducted on the dopamine, opioid, and immune systems as mediators of ketamine's antidepressant effect. For example, ketamine may work in part by decreasing the pro-inflammatory cytokines interleukin-1β, interleukin-6, and tumor necrosis factor. Decreased levels of these cytokines would, in turn, disinhibit pro-plasticity pathways. However, the extant research on ketamine and cytokines remains in early stages and findings contradict one another. Ketamine may also induce epigenetic changes that may be integral to its antidepressant effect (Murrough et al., 2017).

To develop a more complete portrait of the neurobiology of depression and its potential treatments, researchers will seek to integrate findings about the glutamate,

monoamine, opioid, and immune systems with those on regional alterations in brain activity. At this stage, it remains unknown if depression will be found to have a single final common pathway of origin. Instead, depression may in fact represent multiple diseases, with distinct phenotypes that require different treatments. Research on ketamine represents an important tool in tackling these questions. In the meantime, ketamine itself has the potential to be developed into a promising new treatment for depressed patients.

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